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RESEARCH ARTICLE

Botany

Herbarium specimens reveal herbivory patterns across the genus *Cucurbita*

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Abstract

Premise: Quantifying how closely related plant species differ in susceptibility to insect herbivory is important for understanding the variation in evolutionary pressures on plant functional traits. However, empirically measuring *in situ* variation in herbivory spanning the geographic range of a plant–insect complex is logistically difficult. Recently, new methods have been developed using herbarium specimens to investigate patterns in plant–insect symbioses across large geographic scales. Such investigations provide insights into how accelerated anthropogenic changes may impact plant–insect interactions that are of ecological or agricultural importance.

Methods: Here, we analyze 274 pressed herbarium samples to investigate variation in herbivory damage in 13 different species of the economically important plant genus *Cucurbita* (Cucurbitaceae). This collection is composed of specimens of wild, undomesticated *Cucurbita* that were collected from across their native range, and *Cucurbita* cultivars collected from both within their native range and from locations where they have been introduced for agriculture in temperate North America.

Results: Herbivory is common on individuals of all *Cucurbita* species collected throughout their geographic ranges. However, estimates of herbivory varied considerably among individuals, with mesophytic species accruing more insect damage than xerophytic species, and wild specimens having more herbivory than specimens collected from human-managed habitats.

Conclusions: Our study suggests that long-term evolutionary changes in habitat from xeric to mesic climates and wild to human-managed habitats may mediate the levels of herbivory pressure from coevolved herbivores. Future investigations into the potential factors that contribute to herbivory may inform the management of domesticated crop plants and their insect herbivores.

KEYWORDS

Acalymma, coevolution, cucumber beetle, *Cucurbita*, *Erwinia tracheiphila*, herbarium, herbivory, plant-herbivore interactions, pumpkin, squash

Herbaria were originally developed to support research primarily related to plant morphology and taxonomy, later expanding to genetic studies investigating phylogeny and global change biology such as phenology. For centuries, the use of herbarium specimens was largely restricted to studies on these topics. Only recently have methods been developed to also gather ecological information from herbarium specimens (Heberling et al., 2019; Davis, 2023). Over the past few years, research using herbarium specimens has illuminated human influences on plant ecological interactions with insect herbivores, insect pollinators, and microbial associates (Daru et al., 2018; Lughadha et al., 2018; Meineke et al., 2018a, b; Meineke and Davies, 2018). These collections have also been used to quantify how the increasing global footprint of human activities is changing geographic ranges, population sizes, and species interactions of many plant species (Lughadha et al., 2018; Meineke and Davies, 2018; Lang et al., 2019).

Specimens of domesticated crop plants and their wild relatives from herbaria likely contain ecological information for species that are particularly important to human food systems, although this remains largely unexplored.

The plant genus *Cucurbita* (Cucurbitaceae) is an ecologically and agriculturally important model for understanding plant–insect interactions (Metcalf, 1979; Metcalf and Lampman, 1989; Shapiro and Mauck, 2018) and includes pumpkins, gourds, and squashes. Most experimental work on plant–insect ecological interactions involving *Cucurbita* species has been conducted with cultivated populations in their introduced ranges and is notably concentrated in the midwestern and northeastern United States (Metcalf and Lampman, 1989; Sasu et al., 2009; Sasu et al., 2010; Shapiro and Mauck, 2018). Furthermore, very little is known about variation in plant–insect ecological interactions among *Cucurbita* species across their native ranges in the American tropics and subtropics (Kates et al., 2017). Interactions between *Cucurbita* and its coevolved insect herbivores are especially important with regards to agriculture. We postulated that plants grown in regions where *Cucurbita* are naturally found in the wild might have higher levels of herbivory than those grown in regions outside this area, where *Cucurbita* species were spread by humans for agriculture or other purposes. *Cucurbita* have evolved to inhabit a wide range of climatic conditions, from arid to tropical, and via dispersal (by human or nonhuman vectors) they have become common throughout the global tropics and subtropics (Chomicki et al., 2020).

The species that comprise *Cucurbita* fall into two phylogenetic groups. There are six xerophytic (dry-adapted) perennial species native to arid areas in the southwestern United States and northwestern Mexico, and eight species of mesophytic (neither dry nor wet adapted) annuals native to an area spanning the southeastern United States through South America (Nee, 1990; Kates et al., 2017). Five of the mesophytic species have been domesticated for agriculture and remain ecologically and economically important throughout their native range (Nee, 1990; Sanjur et al., 2002; Piperno and Stothert, 2003; Kates et al., 2017). Recent phylogenomic dating using 44 nuclear markers identified that the xerophytic species represent the earliest diverging lineages, and the mesophytic *Cucurbita* represent more recently diverged lineages resulting from a radiation that occurred ~7 million years ago (Schaefer et al., 2009; Kates et al., 2017).

All *Cucurbita* species produce antiherbivory secondary metabolites called "cucurbitacins," which are among the most bitter compounds ever characterized (Horie et al., 2007). Cucurbitacins are effective deterrents for nearly all insect and mammalian herbivores (Chambliss and Jones, 1966; Metcalf, 1979; Metcalf and Lampman, 1989), except for a small subset of highly coevolved leaf beetles in the genus *Acalymma* (Coleoptera: Chrysomelidae: Luperini: Diabroticina) (Andrews et al., 2007; Brzozowski et al., 2019; Brzozowski et al., 2020). These beetles are among the only animals that can detoxify cucurbitacins and consume *Cucurbita* tissues, and all ~70 *Acalymma* species are obligately dependent on *Cucurbita* host plants in all life stages. While some *Diabrotica* and *Epilachna* species also consume *Cucurbita* tissue, these beetles are polyphagous and feed on other plant species (Carrol and Hoffman, 1980; Eben and Gamez-Virues, 2007). The vast majority of chewing herbivory on *Cucurbita* is by *Acalymma* beetles (Du et al., 2008; Hladun and Adler, 2009). For these beetles, cucurbitacins act as arrestants and feeding stimulants (Barber, 1946; Munroe and Smith, 1980; Samuelson, 1994; McCloud et al., 1995; Eben and Gamez-Virues, 2007; Gillespie et al., 2008; Eben and Espinosa de los Monteros, 2013). The ability of *Acalymma* species to metabolize cucurbitacins, and the obligate dependence of *Acalymma* beetles on *Cucurbita* host plants suggests this group of beetles have likely exerted important selective pressures on *Cucurbita* species during the estimated ~4 million years of the coevolution between *Cucurbita* and *Acalymma* species (Metcalf, 1979; Metcalf and Lampman, 1989; Andrews et al., 2007; Gillespie et al., 2008; Eben and Espinosa de los Monteros, 2013). This coevolved beetle species has emerged into temperate eastern North America concurrent with the introduction of *Cucurbita* host plants into this region for agriculture (Kistler et al., 2015; Lopez-Uribe et al., 2016), and now exists in such high population levels that it is the most economically important cucurbit herbivore throughout northeastern North America (Munroe and Smith, 1980; Cavanagh et al., 2009; Brzozowski et al., 2016; Haber et al., 2021).

Despite the economic and ecological importance of *Cucurbita* species, variation in herbivory between species, over time and across space is poorly understood. For example, one puzzling trait of the *Cucurbita-Acalymma* coevolutionary complex is that *A. vittatum* transmits the fatal bacterial wilt, *Erwinia tracheiphila* (Shapiro et al., 2014) but only in temperate eastern North America (Munroe and Smith, 1980; Shapiro et al., 2015; Shapiro et al., 2016; Shapiro et al., 2018a, b). *Erwinia tracheiphila* does not occur in South America or Mesoamerica, which is the evolutionary center of origin and diversification for both *Cucurbita* and *Acalymma* (Shapiro et al., 2016; Shapiro et al., 2018b). The relative paucity of knowledge about plant–insect interactions throughout the native range of both partners constrains our ability to fully understand why this pathogen has such a restricted distribution, and whether other host plant populations may be at risk. It is possible that variation in herbivory may be one of the factors affecting the current distribution of *E. tracheiphila*. *Acalymma vittatum* exhibits a feeding preference for symptomatic, wilting plants infected with *E. tracheiphila* (Shapiro et al., 2012). The expansion of *A. vittatum* into the newly occupied geographic range of its coevolved *Cucurbita* host in the northeastern part of North America has been associated with increased levels of herbivory (Munroe and Smith, 1980; Krysan and Miller, 1986; Cavanagh et al., 2009; Haber et al., 2021). Because *A. vittatum* has followed cultivated *Cucurbita* host plants into their introduced range, causing severe crop damage in this region, we compared levels of herbivory damage on plants collected from this introduced region where *E. tracheiphila* exists to those from plants collected outside this region.

Another trait of particular interest is the classification of *Cucurbita* based on water usage adaptations. This trait may be particularly relevant to understanding the factors driving evolutionary interactions between *Cucurbita* and their coevolved herbivores. For example, in more arid environments, plants may experience less herbivory because their defense adaptations to reduce tissue loss are driven by the need to conserve water and resources (Cunningham et al., 1999; Hanley et al., 2007). The growth-differentiation hypothesis postulates that plant growth is slowed in environments where resources are limited and resources are allocated to the differentiation of cells into specialized structures, i.e., those used for defense (Herms and Mattson, 1992). Based on this theory, we predicted that species that have adapted to conditions of higher water availability would have significantly higher levels of herbivory damage compared to species that evolved for low water-availability conditions. Previous studies have suggested that the transition of *Cucurbita* into mesophytic habitats was followed by rapid species diversification (Kates et al., 2017). We sought to investigate how the long-term evolutionary pressures faced by species classified as either xerophytic or mesophytic may have influenced herbivory levels on *Cucurbita*. While local environmental conditions, such as the intensity and frequency of precipitation, may influence the level of herbivory on individual plant specimens (Howe et al., 1976), our focus was on detecting adaptive differences between species.

Here, we quantify foliar beetle herbivory on the collection of all *Cucurbita* spp. at the Harvard University Herbaria, which houses *Cucurbita* specimens collected throughout the Neotropics and subtropics where *Cucurbita* are native, and in temperate parts of the Americas where cultivated *Cucurbita* have been introduced for agriculture. We use these data to contrast patterns of herbivory on domesticated *Cucurbita* vs. wild relatives and to examine how herbivory varies spatiotemporally. We hypothesized that specimens from mesophytic taxa would have significantly higher levels of herbivory damage compared to xerophytic species. We also postulated that specimens collected from the wild would have higher levels of herbivory damage than specimens collected from managed gardens. This study establishes a "proof of principle" that herbarium specimens can be used to inform investigations into how human activities related to agriculture may be altering plant–insect ecological interactions that are relevant to agricultural production and food security (Munroe and Smith, 1980, Krysan and Miller, 1986).

MATERIALS AND METHODS

Specimen overview

We quantified herbivory on all 274 *Cucurbita* samples held in the Harvard University Herbaria and recorded the label metadata for each specimen (Appendix S1). For some samples, species taxonomy has changed since the original collection. In these cases, the current taxonomy following Kates et al. (2017) was applied. Both the original taxonomic assignments given by the collector and the updated taxonomic assignments are provided in Appendix S1. In other instances, label data were incomplete for the location or date collected. For example, most of the samples were collected before handheld Global Positioning System (GPS) units were widely available, so the location is often given relative to local roads, rivers, or other landmarks. In these cases, latitude and longitude information was approximated based on the location information provided. Location information was used to label samples as being in the geographic region where bacterial wilt disease is present (northeastern and midwestern North America) or outside of the region where the disease is present. Specimens were also coded to record whether they were likely collected from a human-managed garden or from a wild, unmanaged population. However, because the specimens were collected by many different collectors, most of whom did not make notes as to the origin of the specimen, we were unable to distinguish the specimens from temperate regions that were "escaped volunteers" as opposed to plants cultivated in gardens. All samples collected from temperate regions were assigned as "garden" because *Cucurbita* do not naturally occur in temperate climate zones, although we recognize that some of the garden collections could be cultivated varieties that escaped agricultural settings and are growing as volunteers.

Specimen distribution

Collection dates for *Cucurbita* specimens in the Harvard University Herbaria span 181 years, from 1835–2016. Sample locations span from southern Argentina to the northeastern United States and include three specimens collected in the Caribbean islands (Figure 1; Appendix S2, Table S1). Most samples (189 out of 274) were collected from Central and North America (north of the Panama Canal), and only 64 samples were collected from South America (south of the Panama Canal). The lower number of specimens from South America, where several *Cucurbita* species originate and remain culturally and economically important (Sanjur et al., 2002; Piperno and Stothert, 2003), possibly reflects a bias towards collecting in areas closer to the Harvard University Herbaria (Daru et al., 2017).

Out of the 274 total samples, 62 were domesticated plants grown in a garden setting. Of the garden samples, 49 were collected in temperate northeastern North America, where wild *Cucurbita* does not naturally occur, but domesticated varieties have been introduced for agriculture. The remaining 13 garden samples were collected in the American tropics and subtropics, where undomesticated *Cucurbita* wild relatives co-occur with domesticates. The three Caribbean samples were all *Cucurbita pepo* collected from gardens.

FIGURE 1 Map of the geographic distribution of *Cucurbita* specimens in the Harvard University Herbaria Collection with corresponding levels of herbivory damage shows that herbivory exists throughout the geographic range of the genera in the Americas. Different colors correspond to different *Cucurbita* species and larger sized points indicate more severe herbivory on the specimen.

The two most abundant species in the collection were *Cucurbita foetidissima* and *C. pepo*, both of which had from 70–80 samples (Figure 1). *Cucurbita ecuadorensis* was the least common species in the collection, with only two specimens. Six out of eight total specimens identified as *Cucurbita okeechobeenesis* were collected from the eastern coast of Mexico, but these specimens are most likely misidentified because this species is rare, endangered, and endemic to Florida, in the United States (Kates, 2019). As such, these samples were not included in the analysis. This misidentification suggests some taxonomic uncertainty in species identification. *Cucurbita* species are all closely related, and some mesophytic species do not have diagnostic foliar morphological characteristics. Many samples also lack floral reproductive tissues, which could provide more definitive taxonomic assignments (Chomicki and Renner, 2015). In these cases, only the use of high-resolution molecular markers will likely help in correctly determining the taxonomy assignments of the dried herbarium specimens (Agrawal and Fishbein, 2008; Chomicki and Renner, 2015).

Herbivory quantification

For all samples, foliar herbivory was quantified following protocols described in Meineke et al. (2018b). Foliar herbivory damage was quantified using a grid matching the size of standard herbarium sheets (41 × 25 cm) with 40 numbered 5 × 5 cm boxes. Random numbers were used to select five boxes within the 40-cell grid that contained some foliage. The foliage within each of the five randomly selected boxes were visually inspected using a dissecting microscope (10× magnification) for the presence or absence of herbivory damage. If there was any herbivore damage on the specimen in the selected box, this was recorded as a binary (presence/absence) trait. The total number of boxes out of five with herbivory was then summed to produce a score from zero to five with 'zero'

signifying none of the five boxes had herbivore damage and 'five' signifying that all five boxes had herbivore damage.

Chewing damage, from herbivores with mandibles, presents as jagged or smooth-edged holes that measure from 1 to 5 mm in diameter, and destroys both the mesophyll and the epidermis. Chewing damage from *Acalymma* leaf beetles often produces a pattern of small holes in the leaf because they avoid consuming the heavily lignified vasculature tissue around the xylem and phloem tubes (a pattern of damage referred to as "skeletonization"). In rare cases, we also found apparent damage from leaf miners, which is characterized by a thin (approximately 1 mm) path of dead epidermis cells signifying that herbivores have consumed the mesophyll but not the epidermis. We scored chewing and mining herbivory separately. This scoring system resulted in two separate scores from zero to five for chewing and leaf mining damage per specimen.

One challenge in quantifying herbivory on herbarium specimens is distinguishing between natural herbivory (precollection) and herbivory damage that the samples received during storage (postcollection). In a previous study, Meineke et al. (2018a) found that precollection herbivory on the leaves of some plant taxa can be differentiated by the presence of a thin, darkened outline around the damaged area, indicating the plant was still alive when the herbivory caused localized cell death. Postcollection herbivory or storage-related damage is inferred if localized cell death does not occur around the damaged area (Meineke et al., 2018a). We found the same precollection vs. postcollection leaf damage morphologies on *Cucurbita* and therefore applied these same methods for distinguishing precollection herbivory. However, we could not assess herbivory on flowers, even though beetles also consume floral tissues (Anderson and Metcalf, 1986; Anderson, 1987; Metcalf and Lampman, 1990; Shapiro et al., 2012), because it was not possible to differentiate precollection insect herbivory from postcollection damage on delicate *Cucurbita* flowers.

Statistical analyses

Chewing damage was present on 183 (67%) of the total 268 specimens analyzed. Only two specimens displayed mining damage. Because there was so little damage from mining herbivores, and leaf mining insects very rarely attack *Cucurbita*, only the damage from chewing herbivores was included in our statistical analyses.

We built a series of Bayesian models with the Bayesian Regression Models using 'Stan' (brms) package in R (Gelman et al., 2015; Burkner, 2017, 2018) to explore the effects of time, space, and environmental conditions on insect herbivory. In all models, the response was the total number of boxes with herbivory standardized by the total number of boxes scored (five boxes, see above). Effects of each predictor were estimated as the mean and 95% credibility intervals from posterior distributions. Predictor variables were scaled and centered at zero, and effect sizes with a mean and 95% credibility intervals (CI) that did not include zero were considered statistically important. A zero-inflated binomial error structure with default priors was specified for all models. The models were defined as:

$$\text{grid cells with chewing damage} \sim \text{overdispersed binomial}(p_{ij}, n)$$

$$\text{logit}(p_{ij})\_ = a + \beta_1 \text{year}_{ij} + \beta_2 \text{water\_needs}_{ij} + \beta_3 \text{growing\_condition}_{ij} + \beta_4 \text{ disease\_incidence\_zone}_{ij} + \beta_5 \text{latitude}_{ij} + \beta_6 \text{longitude}_{ij} + \beta_7 \text{ precipitation}_{ij} + u_i$$

where grid cells with chewing damage is the number of grid cells with chewing damage by leaf beetle herbivores p on specimen i from species j, and n is a constant representing the number of grid cells examined on each specimen. We model logit(p_{ij}) as a function of a, the intercept, year collected, species water needs (xerophytic or mesophytic), the growing condition (garden vs. wild), the disease incidence zone, latitude, longitude, precipitation, and u_i, which is a grouping factor (random effects) of phylogenetic position. Rhat is the Gelman-Rubin statistic which signifies the potential scale reduction. Rhat values of 1 indicated that all models converged.

Our main model includes all specimens correctly identified ($N = 268$) (Appendix S2, Table S2). We accounted for species-level effects on herbivory by including phylogenetic relatedness as a random effect. Phylogenetic relatedness among *Cucurbita* species was accounted for using a correlation matrix built from the genus phylogeny from Kates et al. (2017) (Appendix S3) and the methods outlined in Turcotte et al. (2014). Phylogenetic effects in the fitted model were estimated as the intraclass correlation (equivalent to Lynch's lambda [Lynch, 1991]) using the "hypothesis" function in *brms*. We then created subsets of the data to further explore model robustness; a description of the additional models including data subsets can be found in Appendix S2.

In our classification, we assigned specimen collected from temperate latitudes as coming from human-introduced environments (the growing condition of "garden"). To investigate whether this confounded separate estimates for the effects of growing condition and latitude, we examined generalized variance inflection factors (GVIF) using the 'car' package in R. We also assigned precipitation values for each specimen using the WorldClim data set, which contains 30-year normal climate data from 1970 to

2017 (Fick and Hijmans, 2017). Because our data set included specimens dated as early as 1835 and the climate data set is from 1970, the analysis provides us with only a general approximation of how precipitation might have affected herbivory levels.

RESULTS

Statistical modeling

We found that chewing herbivory was common on *Cucurbita* specimens across all species examined. Bayesian R-squared (R^2) values ranged from 0.03–0.31 (Appendix S4). The calculation of generalized variance inflation factors (GVIFs) revealed that none of the factors analyzed had GVIF values greater than 4 (Appendix S5). According to previous studies (Fox and Monette, 2012; Gómez et al., 2016) a GVIF ≥10 indicates the potential for collinearity.

In the model including all species, herbivory did not vary over the 181-year timespan (Appendix S2, Table S1; Appendix S6, Figures S1–S2; Appendix S7, Figure S1), or between the disease incidence regions (Figure 2; Appendix S8, Figure S1). Latitude and longitude were not strong predictors of herbivory damage (Appendix S9, Figure S1), suggesting that insect herbivory is common throughout the *Cucurbita* geographic range where wild and domesticated genotypes occur. However, as predicted, mesophytic species had a ~65% increase in the probability of observed chewing damage compared to xerophytic species; no individual species drove this trend (Figures 2–3; Appendix S3, Tables S1–S2; Appendix S10, Figure S1). In addition, wild-collected specimens displayed more herbivory than specimens collected from gardens, where wild specimens had a 69% increase in the likelihood of chewing damage compared to specimens collected from gardens (Figures 2 and 4; Appendix S2, Table S2; Appendix S11, Figure S1). We also found a small, but detectable, relationship between phylogenetic relatedness and herbivory intensity (Appendix S12, Figure S1). When comparing between predictors, growing conditions had the strongest effect on herbivory; specimens collected from wild conditions accumulated more herbivory (Appendix S2, Table S2).

In our analysis of individual species, we found that only the model for *Cucurbita agyrosperma* showed that the growing conditions variable was a strong predictor of herbivory. However, we were unable to perform model analysis on all individual species, because an even distribution of species did not exist in the collection; some species had higher numbers of specimens compared to others.

DISCUSSION

Herbivory by mandibulate, chewing herbivores was common on herbarium specimens from all *Cucurbita* species, and across specimens from this genus gathered throughout tropical, subtropical, and temperate America over a 181-year collection period. We found that mesophytic *Cucurbita* species accrued more herbivory damage than xerophytic species. We further found that specimens collected from the wild experienced more herbivory than those grown under cultivation. The latter result should be interpreted cautiously because the provenance of specimens as garden- or wild-habitat is uncertain in some cases (see below). Nonetheless, our results support suggestions that anthropogenic

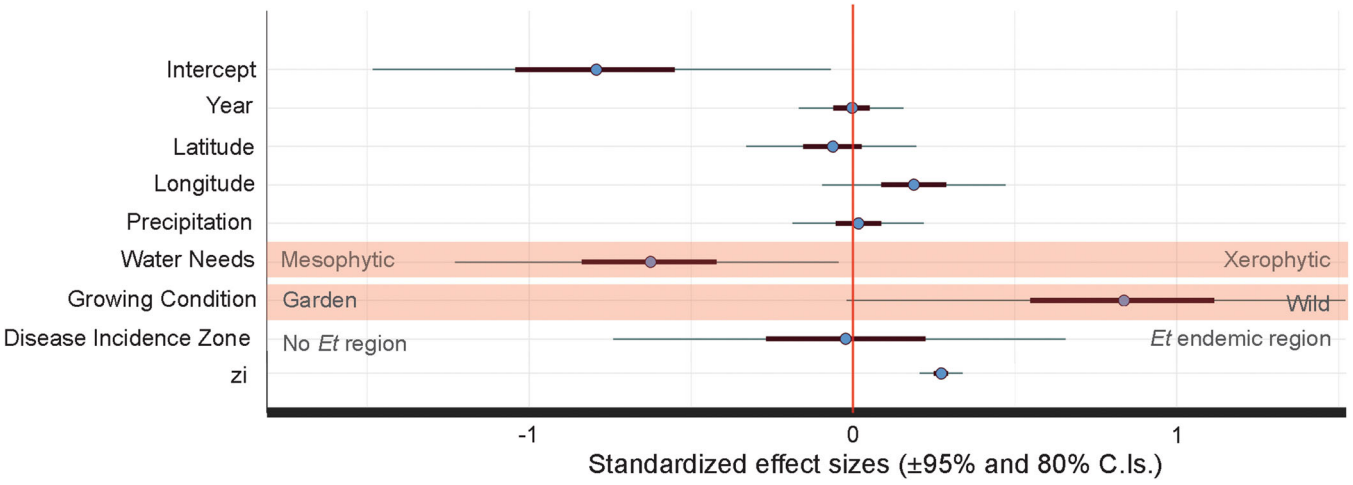

FIGURE 2 Model estimates showing the effects of time, space, plant characteristics, and environmental variables on herbivory damage to *Cucurbita* plants. Bold lines represent 80% credibility intervals and narrow lines represent 95% credibility intervals. The blue circle represents β_{avg}, which is the estimated average effect on herbivory. Shading highlights the interaction term between herbivory damage and the water needs of the plant specimens (xerophytic vs. mesophytic) and the growing conditions of the plant specimens (wild vs. garden). Values of each variable were scaled prior to analysis and thus β_{avg} can be directly compared across model predictors.

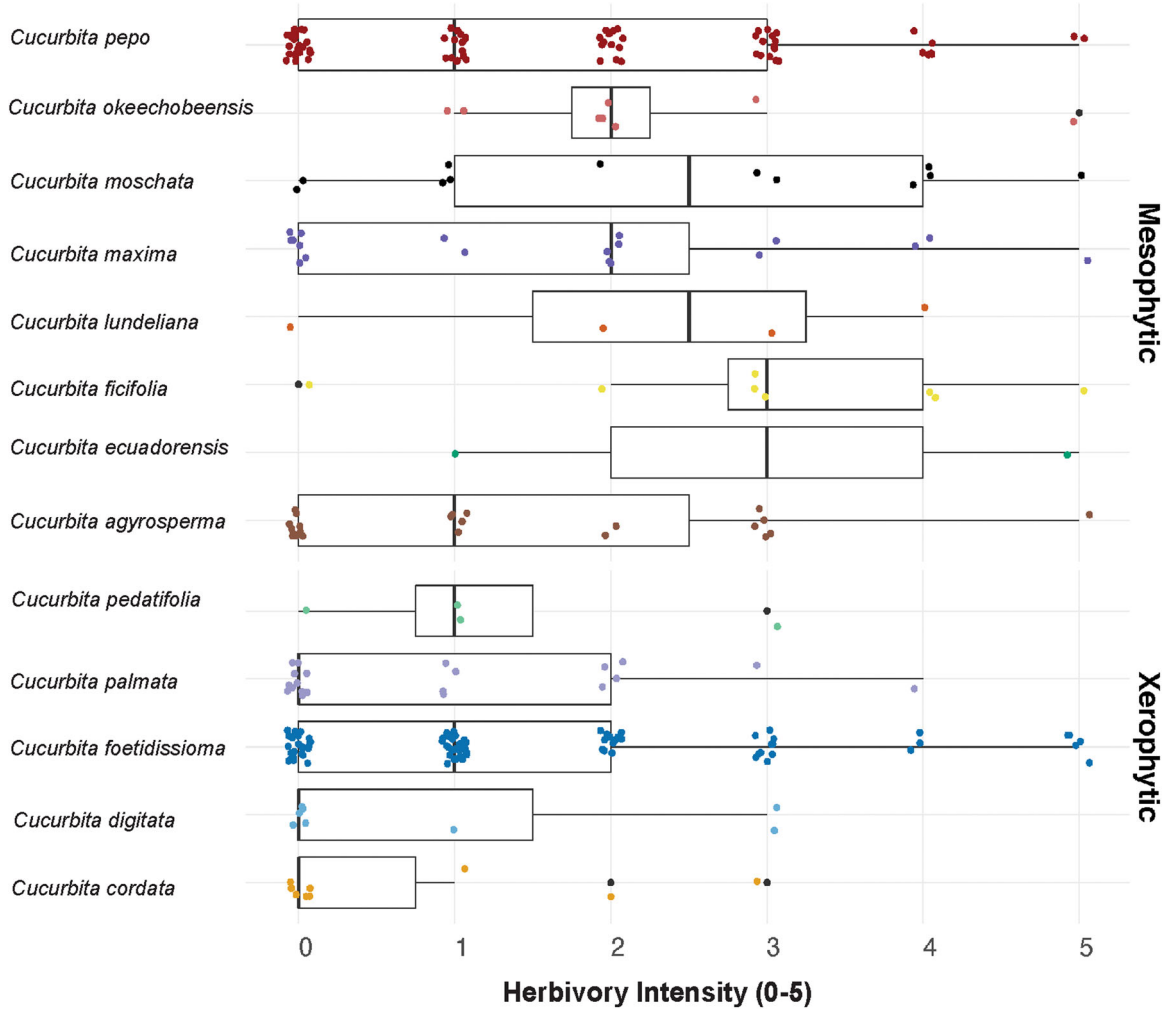

FIGURE 3 Water preference is a strong predictor of herbivory where mesophytic species have higher levels of herbivory damage compared to xerophytic species. Individual dots are colored according to species and represent individual samples in the Harvard University Herbaria Collection. Samples were scored on a scale of 0–5 where zero indicates little herbivory damage and five represents the highest level of herbivory damage.

changes, perhaps from the domestication process or movement of domesticated plants into new geographic areas, may have influenced plant–insect herbivore interactions within this group of food plants.

Phylogenetic analyses demonstrate that *Cucurbita* originated in xeric habitats in northwestern Mexico and the southwestern part of the United States; mesophytic species evolved later as a result of radiation into wetter habitats throughout the American tropics and subtropics ~7 million years ago (Hurd et al., 1971; Schaefer et al., 2009; Kates et al., 2017). Our finding that *Cucurbita* specimens from mesophytic habitats have higher levels of herbivory damage suggests their evolutionary transition from dry to moderate habitats may have affected interactions with coevolved herbivores. Evolutionary transitions to new habitats can provide many advantages to plants, including the possibility of escaping from herbivores (Agrawal, 2008). However, contrary to this escape scenario, we find that mesophytic *Cucurbita* species display more herbivory damage than xerophytic species. Our results suggest that while *Cucurbita* have spread successfully throughout subtropical regions in the Americas, escape from herbivory was not likely a facilitating factor in this range expansion (Bang and Faeth, 2011). An alternative explanation for the pattern we observed is that desert arid environments exhibit conditions that make plants subject to less herbivore pressure compared to those in mesic habitats (Cunningham et al., 1999; Hanley et al., 2007).

In the overall model that includes all species, specimens collected from garden habitats had lower levels of herbivory compared to those collected from wild habitats. In our analysis, we accounted for the growing conditions of the plants to the extent that was possible by categorizing specimens as collected from gardens or wild habitats. However, there is some uncertainty about whether all tropical plants labeled as "wild" are actually wild—some of them could be from gardens but not labeled as such. It is also possible that some specimens that were collected from outside the native range of *Cucurbita* and thus labeled as coming from human-managed gardens in our study might

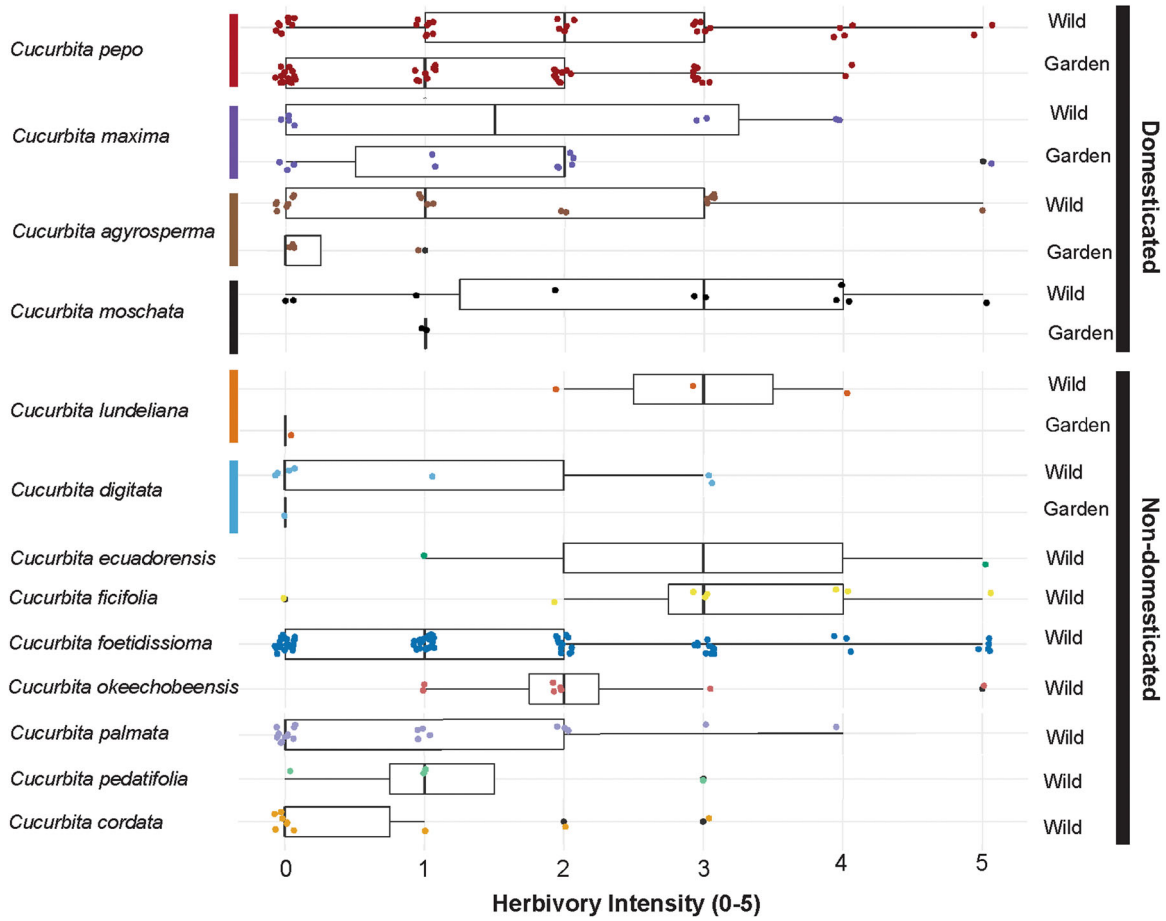

FIGURE 4 Specimens collected from wild settings displayed more herbivory than specimens collected from gardens. Individual dots are colored according to species and grouped into domesticated and nondomesticated species types. Herbivory intensity was scored on a scale of 0–5 for each specimen where zero indicates little herbivory damage and five represents the highest level of herbivory damage. When possible, the median of herbivory was plotted (represented by the horizontal line in the center of the box).

actually be escaped volunteers. In models of individual species, we identified a strong effect of growing conditions for *C. agyrosperma* and not for other species, although low sample sizes for the other species might have driven this pattern. Models that included all species thus suggest that cultivation does indeed affect herbivory patterns in *Cucurbita*, but future investigations of collections that include more specimens per species and more domesticated specimens from the tropics would help determine if our conclusions are robust across species and the entire range of the genus.

Five mesophytic species were domesticated for agriculture within the past 10,000 years (Kates et al., 2017; Chomicki et al., 2020). The finding that herbivory levels are higher in wild specimens than in garden-collected specimens suggests two non-mutually exclusive potential mechanisms. First, it is possible that domesticates are less damaged by coevolved herbivores because crop breeding has reduced the amount of cucurbitacins in domesticated cultivars. Rather than being deterred by defensive compounds like most herbivores, the coevolved beetles that feed on *Cucurbita* are attracted to cucurbitacins and selectively feed on them. *Cucurbita* have evolved to tolerate some amount of herbivory from the few coevolved leaf beetle herbivores that are able to consume foliage containing cucurbitacins (Ferguson et al., 1983; Strauss and Agrawal, 1999). *Cucurbita* plants grown in gardens may have lower cucurbitacin levels because of the process of domestication for agriculture (Brzozowski et al., 2019), reducing herbivory pressure from leaf beetle herbivores. Second, plants grown in gardens may experience reduced damage because of anthropogenic interventions such as insecticides. Because our data set could not distinguish cultivated samples grown in gardens or agricultural fields from domesticates that are growing as weeds, experiments testing beetle attraction to, and herbivory on, wild vs. domesticated *Cucurbita* will be necessary to distinguish these two hypotheses.

We also investigated the extent to which patterns in herbivory may covary with the incidence of cucurbit bacterial wilt caused by *Erwinia tracheiphila*. Our results indicate that herbivory is ubiquitous throughout the Americas, including in regions outside the region where this obligately herbivore-transmitted pathogen occurs

(Figure 2). These findings support previous investigations that found agricultural intensification and crop plant introductions—and not the geographic distribution of the beetle vector—underlie the recent emergence of this pathogen (Shapiro et al., 2016; Shapiro et al., 2018b). Our finding that herbivory is ubiquitous throughout the time and geographic locations surveyed provides evidence that feeding frequency from obligate beetle vectors does not restrict the geographic distribution of the disease.

One challenge that arises using herbarium specimens is that collection biases can arise across space, time, and phylogeny (Daru et al., 2018). For instance, taxonomists may try to select plants with less herbivory for preservation over plants with more herbivory. However, as long as bias does not shift systematically across axes of interest (e.g., time, latitude), herbarium specimens can still accurately represent levels of damage among specimens (Meineke and Daru, 2021). The digital collection available from the Harvard University Herbaria does not currently include all the *Cucurbita* samples in the collection, limiting our ability to collect additional specimen data, such as specimen size, which could have improved the predictive power of our models. Continued digitization of such collections would be of immense importance for improving studies like the one we present here (Hedrick et al., 2020). The geographic separation of xerophytic vs. mesophytic plants opens the possibility that an alternative factor might explain the differences in herbivory levels. However, for this trait, geographic separation is expected as the classification relates to the water preferences of the plants, which are regionally distinct.

CONCLUSIONS

Our study demonstrates the immense value of using herbarium specimens for characterizing plant-biotic evolutionary interactions. We demonstrate that evolutionary history, and potentially growing conditions, systematically determine the level of damage experienced by *Cucurbita* species, a clade that has been highly shaped by its interactions with specialized insect herbivores. However, the challenges implicit in studies like the one we present should not be underestimated. *Cucurbita* species, in particular, are morphologically similar and difficult to identify. For instance, the species delimitations and taxonomy of *Cucurbita* have changed several times during the last several decades, and it is possible that some specimens in the Harvard University Herbaria collection have been assigned names based on outdated nomenclature. Time and funding permitting, additional data using molecular barcode markers would be valuable for classifying specimens. Nonetheless, our study demonstrates that herbarium specimens represent a rich source of species interactions data that can provide unique insights spanning an entire widely distributed plant genus for which herbivory data are sparse across space and time.

AUTHOR CONTRIBUTIONS

L.J., L.S., and E.M. conceived of the study. L.J. performed the data collection of herbarium specimens. L.J., E.M., C.D., and J.D. developed analytical methods. L.J. and E.M. analyzed data and all authors interpreted data. L.J., L.S., E.M., and N.P. wrote the first draft of the manuscript, and all authors revised the manuscript.

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DATA AVAILABILITY STATEMENT

All data is contained in the Github repository: https://github.com/laurajenny/Cucurbita_herbaria_sup.git.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Data collected from the Harvard University Herbarium during this experiment.

Appendix S2. Summary of specimen distribution and Bayesian models.

Appendix S3. Correlation matrix file for phylogeny of *Cucurbita*.

Appendix S4. *R*-squared values for Bayesian models.

Appendix S5. Results of the generalized variance inflation factors analysis.

Appendix S6. Distribution of specimens over time and space.

Appendix S7. Distribution of specimens over time separated by domestication status.

Appendix S8. Geographic distribution of specimens separated by disease state.

Appendix S9. Geographic distribution of wild and garden specimens separated by latitude.

Appendix S10. Geographic distribution of specimens separated by water needs.

Appendix S11. Geographic distribution of specimens separated by growing condition.

Appendix S12. Phylogenic tree of species relatedness and herbivory intensity.

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